Soil Organic Matter as a Natural Chelating Material

Part 2: The Occurrence and Importance of Paradoxical Concentration Effects in Biological Systems

To understand how organic matter overcomes or counteracts certain toxic substances in the soil, it is necessary to understand the nature of the toxic effects themselves.

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THE ACCUMULATION OF insecticides, fungicides, and herbicides in soils and the resulting phytotoxic effects have already posed major problems[52]. That chemical fertilizers greatly increase the toxicity of soils which have been treated with these biocides[52] only increases the seriousness of the situation. Fortunately, however, the adverse effects of biocides can be overcome by supplementing soils with biologically active forms of humus which have a high exchange capacity and supply energy and nutrients. In some cases, this beneficial action may be attributed to the ability of humus to chelate the lead, copper, mercury, zinc, and arsenic in certain biocides. The specific role of soil organic matter as a complexing agent for metals [53] and the concept of the soil solution as a metal-buffer will be discussed in detail at a later date. At present, we will concern ourselves here with paradoxical toxic effects which may have very serious implications with respect to the dangers of biocides. The unique aspect of these paradoxical effects is that toxicity actually increases as the concentration decreases![54] This present paper will discuss paradoxical effects from a broad, comparative biochemical point of view to show that they occur widely throughout nature and what mechanisms are responsible for some of these phenomena. This information will provide a background and perspective for better understanding those paradoxical effects that have been reported in soil-plant systems which will be reviewed in a subsequent publication.

Dose-Response Curves

The important properties of drugs, nutrients, inhibitors, and other biologically active agents include dose, efficacy, toxic level, mode and site of action, and side effects. Some of this information can conveniently be presented in the form of a time-response curve. This shows how much of an effect is produced by a given dose at different periods of time following administration of the agent. Such a curve tells, for example, how soon the effect develops and how long it persists. A dose-response curve, on the other hand, reveals the quantitative relationship between the concentration of the particular agent and the magnitude of the effect.

It is generally assumed that dose-response curves are or should be sigmoid on arithmetic coordinates, and straight lines when plotted logarithmicly (Fig. 1). Such curves are often produced by both growth-enhancing and growth-inhibiting agents where the effect may be directly or inversely related to the concentration of the substance tested. Because these curves are so frequently encountered, they have at times been used to define dose-response relationships. But sigmoid and other curves (Fig. 2) which cannot be represented by single straight lines are also obtained on logarithmic coordinates.[1] In all these cases, however, the effect generally increases with the concentration of the variable.

Other kinds of dose-response curves are also known. So-called oligodynamic substances, such as certain heavy metals, are stimulatory at low concentrations whereas higher levels are toxic (Fig. 3). The opposite effect, which may at first seem incredible, has also been encountered. Here, over a given concentration range, a compound may be more toxic at lower than at higher doses (Figures 3 and 4). This is unusual because inhibition is actually reduced, overcome, reversed, or antagonized by simply adding more of the same inhibitor! In such systems where dose of the toxic factor is the only variable, the antidote for the toxic agent is more of the same agent itself!

In this paper, we will consider these paradoxical effects where toxicity first increases, then decreases, and finally increases again as the concentration of the inhibitor increases. Such effects occur more frequently than one would ordinarily assume. Furthermore, their theoretical and practical importance in biology, medicine, agriculture, and many other fields, has not yet been fully appreciated. We propose to show that paradoxical effects are not isolated phenomena, but are broadly operative and of widespread importance in the biochemistry and physiology of many living systems under many different conditions.

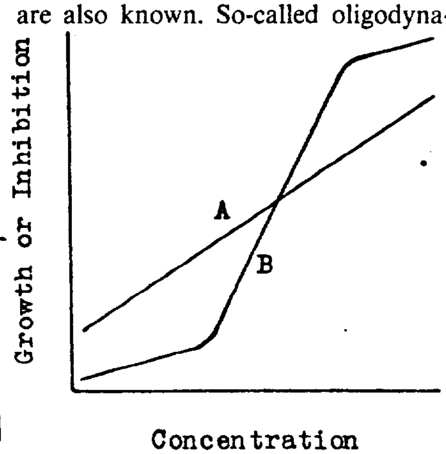


Fig. 1. Dose-response curves showing linearity (A) on logarithmic coordinates and a sigmoid shape (B) when plotted on arithmetic coordinates.

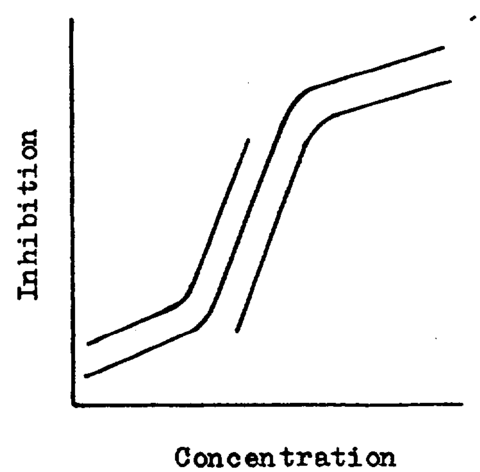


Fig. 2. Three dose-response curves that are not straight lines on a logarithmic grid.[1]

This paper reports part of an extensive survey of paradoxical effects which was made possible by a grant from The Soil and Health Foundation, Emmaus, Pennsylvania.

Example of a Paradoxical Effect

Paradoxical effects appear as dips in dose-response curves. This is illustrated in Fig. 4 which shows the inhibition of *Salmonella typhimurium* by adenine. Note here that 10 micrograms of adenine per milliliter definitely retarded growth whereas twice this concentration and, in some cases, three and even four times as much adenine are less inhibitive. The paradoxical effect occurred, moreover, with each of four different levels of thiamin (which this organism requires for growth) and the dips in all curves appeared at the same concentration of adenine. Some curves showing paradoxical effects in other systems have *two* dips.[3, 50] From a comparative biochemical point of view, it is interesting that 8-hydroxyquinoline (oxine)[4] and certain aminopyrazolopyrimidines,[5, 25] which are structurally somewhat similar to adenine, also produce paradoxical effects in their inhibition of microbial growth.

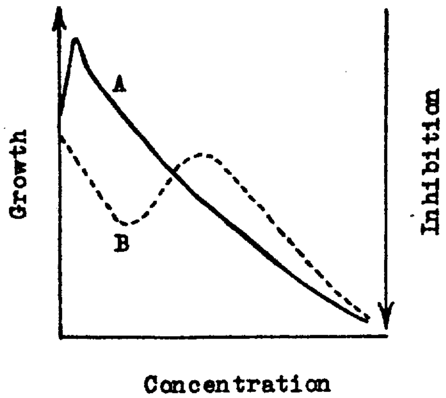


Fig. 3. Curves on arithmetic coordinates showing oligodynamic (A) and paradoxical (B) effects.

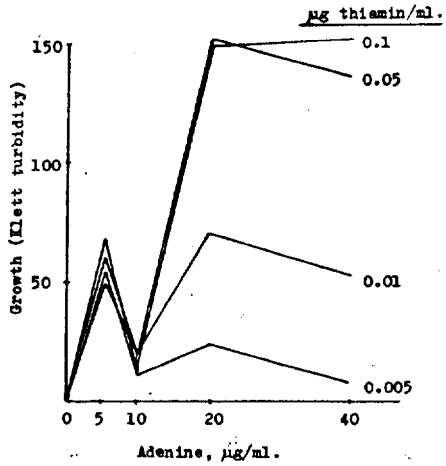
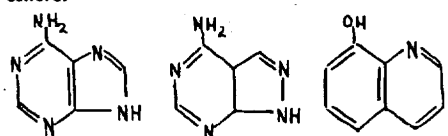


Fig. 4. Inhibition of growth of *S. typhimurium* by adenine after 24 hours. Lightly-inoculated cultures were set up according to a procedure already described.[2] The number on each curve indicates the amount of thiamin in that particular culture.



Adenine 4-Aminopyrazolo-(3, 4-d) pyrimidine Oxine

Terms Used To Designate Paradoxical Effects

Different investigators who have encountered these peculiar concentration phenomena have described them in such terms as paradoxical effect,[6, 7] paradoxical zonal effect,[8] paradoxical zonal susceptibility,[8] paradoxically retarded bacterial action,[8] zone phenomenon,[8, 9, 10] zonal susceptibility,[8] zoning,[10] inversion phenomenon,[11] inversion growth,[12, 13, 14] bimodal dosage response,[14] bimodal curve,[15] polymodal curve,[15] biphasic activity curve,[16] diphasic effect,[51] folded curves,[17] broken lines,[18] second growth stimulation,[19] concentration quenching,[4] phenomenon of the double inhibition maximum,[12] and death zone.[20]

Failure To Recognize Paradoxical Effects

In some cases, paradoxical effects have not been observed because they occurred above or below the concentration range which was tested, or at intermediate concentrations which were not tested.[30, 37] In other cases, where paradoxical effects are clearly evident in data or curves, most authors do not even mention these inconsistencies in discussing their findings. Most readers likewise take no account of dips in dose-response curves or data. It would be surprising, for example, if all 200 compounds tested for antimicrobial and antitumor activity in one particular study gave *only* sigmoid curves as was reported.[21] A review of cancer chemotherapy considered only *one* kind of dose-response where survival curves rose as the concentration increased, but then dropped after a certain dose was exceeded.[22] For both microbial and tumor inhibition some substances definitely exhibit paradoxical effects. In plant nutrition and crop yields, it has been "emphasized that sigmoid curves, or parts of sigmoid curves, are often observed for all the ordinary plant nutrients when the experiments are performed with highest precision; that is, with a very small experimental error and with a growth medium highly deficient in the nutrient in question."[23] However, the very paper which makes this allegation includes two sets of experimental data which clearly show paradoxical effects not mentioned by the authors. These three examples have been selected to show how we are conditioned to think in terms of sigmoid or linear dose-response relationships. So we attribute deviations, when we notice them at all, to experimental error or experimental variability. Although this assumption is often correct, it ignores some problems that will eventually have to be recognized.

The fact that careful statistical treatment of experimental data does not reveal paradoxical effects does not mean that such phenomena do not exist. On the contrary, statistical methods can most effectively prevent recognition of paradoxical effects because the mathematics employed in statistical analyses do not consider these phenomena. With scattered points, statistical methods are used to determine where the *straight line* or *smooth curve* should be drawn. The whole statistical approach assumes that these would be the *ideal* curves. Deviations or irregularities caused by paradoxical effects are treated as experimental errors or experimental variation. It is, therefore, paradoxical that statistics, employed to minimize or avoid mistakes, can be responsible for concealing paradoxical effects.

Are Paradoxical Effects Real?

Because paradoxical effects are so unusual and contrary to most preconceived notions, we will quote from original sources to avoid misinterpreting the reports of investigators who have specifically taken note of irregularities in their data. The following examples have been selected, because they are unequivocal paradoxical effects produced by a wide variety of chemical agents in microbial, plant, and animal systems.

When Pace "noted . . . two optima . . . in the growth curves" of *Amoeba proteus* with increasing concentrations of $MgCl_2$, he "at first . . . thought that this was due to experimental error, but" duplicate experiments with this and several other salts alone and in combination, showed "at first an increase in numbers of amoebae with an increase in salt concentration. This is followed by a decrease in numbers. However, with further increase in concentration the amoebae increase in numbers until a second optimum is reached which is followed by a decrease in numbers of amoeba with increasing salt concentration."[24] Fuerst *et al.* repeated an experiment on the effect of a pyrazolopyrimidine on *Neurospora crassa* eleven times, but always got the same result: "The dose-growth curve went down at first and turned upwards when the concentrations were higher."[25]

Horsfall and Rich discussed the dose-response curves of antifungal 8-quinolinol compounds with "two peaks" where "toxicity . . . increases . . . between the . . . peaks . . . as concentration decreases." They said that "this is easy to write off as experimental error, but it definitely is not. No very certain explanation for this phenomenon exists."[15] Albert, who studied the antibacterial properties of oxine, asked: "Who would expect the" toxic "effect of any biologically active substance to decrease as the concentration is increased? However, a few instances have been found where this does occur."[4] Diamond *et al.* raised two important questions in connection with the effect of tetramethylthiuram disulfide on molds: "(a) How can a single toxic agent pass through two distinct dosage regions, each with its characteristic LD-50 and slope values? (b) How can a material become more toxic on dilution?"[18] Jefferson and Sisco found that "progesterone surprisingly caused an inhibition" of *Aspergillus niger* "at low concentrations and a stimulation at higher ones."[26] *Penicillium ochrochloron,* according to MacMillan *et al.*,[27] was inhibited by intermediate but not by high or low concentrations of $CuSO^4$. After working independently with the same organism, Basu *et al.* concluded: "copper is . . . toxic to" *Penicillum ochro-chloron* "as expected, but . . . through some mechanism harmful doses are absorbed only at certain copper levels of the medium."[19] The effect of urethan on the phagotrophic chrysomonad *Poteriochromonas stipitata* was paradoxical. "Final concentrations of 10 and 50 micromoles of urethan inhibited endogenous respiration, but increased oxygen consumption was observed with 1 and 100 micromoles."[28]

Various paradoxical effects have been reported in higher plants. Ploquin and Ploquin, who studied the effect of boric acid on the germination of wheat seeds, commented: "We wish to direct attention to an observation that we have confirmed by repeating the experiment several times with several groups of 100 seeds: the curve obtained with boric acid exhibits two maxima and these do not seem to be due to experimental error."[29]

The effect of trans-caffeic acid on growth of the *Avena* coleoptile showed "two maxima in a very significant way. In a second experimental series . . . the minimum . . . is even more obvious. It is not possible as yet to give a well-founded explanation for this peculiar concentration effect."[30] "Morin . . . behaves in a diverging way" in its effect on wheat roots in nutrient solution. "In rather low concentrations the growth is distinctly inhibited, whereas somewhat higher concentrations are less inhibitory."[31] With *Avena* "seeds after-ripened for four months . . . the embryos show two responses to gibberellic acid with distinctly different concentration optima . . . If the seed is mature but not after-ripened, the embryos show a response only to the high range of concentrations."[32] In studies on the elongation of wheat roots, "the toxicity of Magnamycin . . . was increased with decreasing concentration . . . The reason for this response is not known."[33] Borst Pauwels found that low levels of potassium iodide inhibited both flax and white clover while higher levels stimulated. "It is interesting," he pointed out, "that the growth . . . was markedly depressed by the lowest dose of iodide, a fact which we are unable to explain."[34]

Thompson and Pace were surprised when they encountered paradoxical effects of sulfite, bisulfite, and sulfate on human and mouse cells in tissue culture. "At present," they wrote, "this phenomenon is not understandable. It is inconceivable that a substance should have a greater toxic effect at a low . . . than at a high concentration."[35] Nevertheless, that is precisely what occurred in their experiments and in the experiments of many others.

"The curve of inhibition" of human prostatic acid phosphatase "by fluoride as a function of fluoride concentration differs from that found with other inhibitors in the occurrence of a point or region of maximum inhibition."[36] There is "an unusual pattern of inhibition, which earlier investigators had evidently missed as a result of the more restricted range of conditions which they employed . . . As the fluoride concentration is increased over a thousand-fold range, the extent of inhibition rises, attains a maximum which . . . may approach 100%, and subsequently falls (generally not to zero in the range of concentration used). A secondary rise of inhibition at still higher fluoride concentrations may or may not be observed . . . The distinctive feature . . . is the decrease of inhibition at high fluoride concentrations."[37] "The protection" of human prostatic phosphatase against thermal denaturation "at varied concentrations of fluoride" also "passes through a maximum. This curve parallels the corresponding inhibition curve."[38]

In studies of the effect of dinitrophenol on the respiration of blowfly thoracic muscle sarcosomes, "the most interesting point . . . is the effect of different concentrations . . . on the esterification of phosphate. Low concentrations . . . sharply decrease the amount of esterified phosphate, but further additions increase it again, until . . . the esterification of phosphate is actually . . . greater than in the absence of dinitrophenol."[39] A paradoxical situation was encountered in the process of assaying 9-fluorhydrocortisone in adrenalectomized dogs. Very small doses of the steroid caused sodium retention, but larger doses caused sodium loss."[40] In the treatment of diabetes, the "paradoxical fact" has been noted "that *excess insulin action can produce hyperglycemia.*"[41] It is "a paradoxical fact that *hypoglycemia begets hyperglycemia.*"[42]

Mechanisms of Paradoxical Effects

The mechanism or mode of action of paradoxical effects has been elucidated for some systems. In certain cases, it is due to different biochemical or physiologic effects that become operative at different concentrations.[40] Or, several enzymes in an integrated biochemical pathway may vary in their susceptibility to an inhibitor or to intermediate products which accumulate as a result of inhibition. Under these conditions, the net activity of the overall system may vary paradoxically as the concentration of the inhibitor increases.[39]

In other cases, the purely quantitative increase of one constituent introduces qualitatively new molecular species which may have entirely different properties from the substance that is added. This can occur in different ways. For example, the above-mentioned paradoxical effects of fluoride,[36, 37, 38] have been attributed to the formation of polymers. The inhibitor is believed to be the dimer

HF_2-which combines with some electropositive group on the enzyme with which the substrate normally associates. "A higher polymer, probably $(HF_2)_2]^{2-}$, also combines with this group, but can be displaced by substrate to some extent; the tetramer, therefore, acts to protect some of the enzyme from the dimer, and inhibition drops below its maximal level at higher fluoride concentrations, where this form appears in significant concentrations."[37]

The formation of new molecular species also occurs in systems where coordination takes place. Metal complexes can be quite different in their biological properties, from free metal ions or ligand molecules alone. Moreover, the biological activities of different metal complexes will vary greatly depending on the metal: ligand ratio and which metals have been coordinated. The following three examples show how such systems can operate paradoxically.

In a strictly chemical system, some curves for the oxidative deamination of glycine show a pronounced dip in the rate of reaction as the concentration of copper is increased in the presence or absence of fixed amounts of citrate, malate, mandelate, salicylate, and resorcinol.[43], [44] With constant amounts of glycine and copper, a paradoxical effect also occurred when the concentration of salicylate[44] or NaOH[45] was increased. In some of these experiments, there were one, two, or three levels of alkali for which the rate of the reaction was maximal.[3] In other words, certain curves had as many as three peaks with two dips in-between. Similar paradoxical effects also occurred in the oxidative deamination of alanine when the concentration of NaOH was increased.[46]

In these systems, the most important factor determining the rate of reaction is the solubility, composition, and stability of the complexes, and all these properties are altered by a change in alkali concentration.[46] As more NaOH is added, the following complexes which contain decreasing amounts of glycine are formed: $[Cu(NH_2\text{-}CH_2\text{-}COO)_4]^{2-}$, $[Cu(NH_2\text{-}CH_2\text{-}COO)_2(OH)_2]^{2-}$, and $[Cu(NH_2\text{-}CH_2\text{-}COO)(OH)_3]^{2-}$.

Finally, only $[Cu(OH)_4]^{2-}$ which contains no glycine at all is produced. The rate of oxidative deamination varies paradoxically because these complexes, which are intimately involved in this reaction, differ greatly in their composition, stability, and solubility.

A somewhat analogous paradoxical effect in a biological system was caused by different calcium:dipicolinic acid complexes. As increasing amounts of calcium were added to a bacterial spore suspension in the presence of a constant concentration of dipicolinic acid, germination was first inhibited, but then stimulated. This was due to the different biological properties of complexes formed in sequence. The free ligand acid, which was originally present and enhanced spore germination, was converted to the 1:2 complex that was inactive. The addition of more calcium, however, converted this 1:2 complex to the 1:1 chelate which, like the free ligand, favored germination.[47]

The paradoxical effect of dithiocarbamyl compounds on the growth and respiration of various fungi has a similar explanation. As the concentration of dithiocarbamate is increased, the toxic 1:1 copper complex is formed first. Beyond this point, the addition of more inhibitor results in less inhibition, because the toxic 1:1 complex is converted to the insoluble 1:2 copper:dithiocarbamate chelate. The further addition of dithiocarbamate again produces inhibition because of the formation of zinc, manganese, iron, and other heavy metal complexes which are less stable than those of copper.[11], [48]

Are Paradoxical Effects Reproducible?

The criterion of reproducibility is often applied to determine whether an effect is real or due to experimental variability or experimental error. It is therefore paradoxical that paradoxical effects are not always reproducible in the ordinary sense. According to Goksoyr who elucidated the mechanism of the paradoxical effects produced by dithiocarbamates: "The degree of inhibition of the 1:1 copper complex is extremely variable in all experiments. The reason for this is not clear."[11] He did feel, however, that the amount and nature of heavy metal contamination, especially copper, in the medium could influence the response of an organism to a dithiocarbamate. He also considered that size of the inoculum might be important in this respect, because chemical contamination is often introduced in this way.

For temperature-induced paradoxical effects on bacterial growth, "conditions are very critical and small changes in the medium composition or presence of residual detergent in the test tubes will influence the experimental results."[49] Moreover, the paradoxical results which were clearly apparent after 24 hours incubation were no longer evident after 48 hours, because all cultures had by then developed maximum turbidity. In addition to substances present in trace amounts, the nitrogen, carbon, and energy sources available to an organism may likewise determine whether paradoxical effects occur.[12], [19] Since pH affects the stability of metal complexes, it is not surprising that paradoxical effects may appear only within certain pH ranges.[13]

The paradoxical effect of progesterone on *Aspergillus niger* was confirmed by repeated experiments which all gave dips in the growth curves. But the shape of these curves varied because maximum inhibition of growth did not always occur at the same concentration of steroid. The dips in the curves therefore, appeared at different points from one experiment to another. The paradoxical effect of adenine on growth of *Salmonella typhimurium*, which has already been described, also depended on a light inoculum and was detectable only in certain media and only after one day incubation. After 48 hours, maximum growth had occurred with all concentrations of adenine. Here, too, replicate experiments often gave curves with dips corresponding to different adenine levels.

One other factor which may determine whether a paradoxical affect will occur is the organism itself. Different organisms and even different strains within the same species vary considerably in their biochemical and physiological behavior. This is illustrated by the fact that dithiocarbamate produced paradoxical effects with some molds, but not others.[12] Also, the paradoxical effect of dipicolinic acid on spore germination was "an exception" which occurred with only one strain of bacteria among many that were studied.[47]

Conclusions

Paradoxical effects have heretofore, not attracted the attention they merit because the reports are scattered throughout many fields. For this reason, most investigators who encountered these phenomena were not aware of similar findings in other disciplines.[50] These effects have therefore, been looked upon as isolated cases. Actually, however, they comprise a distinct *group* of dose-response reactions, each including a concentration range characterized by more inhibition at the lower level. The unique aspect of the paradoxical effect is that inhibition or toxicity is overcome by simply adding more of the original inhibitor or toxic agent, and nothing else. In other words, more of the same thing produces a diameterically opposite effect; that is, a purely quantitative change in one constituent transforms the system qualitatively.

The mechanism or mode of action is unknown for most paradoxical effects but has been elucidated in some cases. Since all biological systems operate via metal complexes, it is not surprising that certain paradoxical effects are due to the formation of a family or series of metal complexes with members which differ in biologic properties. Chemistry is replete with reactions which reverse themselves in the sense that precipitates are solubilized by an excess of the precipitating reagent. Similar phenomena may be involved in those paradoxical effects which are due to metal complexes. Other modes of action, however, are also known.

Paradoxical effects occur more frequently and are more widely distributed throughout biological systems than one would ordinarily assume from the scattered reports. Under certain conditions, such diverse forms as bacteria, protozoa, algae, wheat, and mammals have all exhibited these phenomena when their growth, inhibition, respiration, enzymes, or other vital activities were treated with different concentrations of various agents. Paradoxical effects have been produced by radiation, temperature, mutagenic and carcinogenic chemicals, fluoride, steroid hormones, dextran, detergents, trace metals, herbicides, fungicides, insecticides, germicides, antibiotics, drugs, and a host of other agents. Subsequent articles will discuss each of these groups separately and in detail. Since numerous chemical and physical agents cause paradoxical effects by different mechanisms in many biological systems, these reactions will no doubt become increasingly important in pharmacology, toxicology, chemotherapy, drug idiosyncracies, air pollution, chemical carcinogenesis, fluoridation, fallout, radiation effects, nutrition, biogeochemistry, the weathering of rocks and minerals, soil formation and soil fertility, crop production, and many other areas.

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